Biological hazards
and their tenacity in spices and dried herbs

Name of Presenter	Anneluise Mader and the SPICED Consortium
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This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 312631.

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Outline

1. Introduction
2. Agents and matrices
3. Spiking and detection
4. Tenacity
5. Conclusion and outlook

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Foodborne outbreaks in the EU

Year	Number of foodborne outbreaks	Outbreaks with identified foodstuff
2007	5733	1784
2008	5332	890
2009	5550	977
2010	5262	698
2011	5648	701

efsa
European Food Safety Authority

EFSA

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Outbreaks associated with spices and herbs (Europe, 1973 – 2012)

In total	22 outbreaks
	1821 cases
	>39 hospitalizations
	>1 deaths

Associated agents, cases, hospitalization and deaths

	Outbreaks in %	Cases in %	Hospitalizations in %	Deaths in %
Bacillus cereus	50	24	0	0
Salmonella	27	71	100	100
Clostridium perfringens	23	5	0	0

Mader and Schaarschmidt 2015

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Outbreaks associated with spices and herbs (Europe, 1973 – 2012)

In total	22 outbreaks
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	>1 deaths

Associated agents, cases, hospitalization and deaths

→ Paprika chips outbreak in Germany 1993 excluded!

	Outbreaks in %	Cases in %	Hospitalizations in %	Deaths in %
Bacillus cereus	50 → 52	24 → 54	0 → 0	0 → 0
Salmonella	27 → 24	71 → 36	100 → 100	100 → 100
Clostridium perfringens	23 → 24	5 → 10	0 → 0	0 → 0

Mader and Schaarschmidt 2015

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Agents and matrices in the focus

Agents	Matrices
	
Bacillus spp.	Allspice (Pimento)
Brucella spp	Cinnamon
Clostridium perfringens	Nutmeg
Escherichia coli	Paprika
Listeria monocytogenes	Pepper
Salmonella spp.	
Staphylococcus aureus	Basil
Ricin	Oregano
Staphylococcus aureus enterotoxin B	Parsley

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Spiking of low moisture food

Method	Pro's	Con's	Suitable	Used
Freeze-drying	+ Direct	- Impact on tenacity?	+	✓
Air-drying	+ Natural	- Adhesion to vial (low amount)	~	✓
Matrix (fluid)	+ Direct + Natural	- Clumps - Agents/matrices interferences - a_w changes	~	✓
Matrix (spray)	+ Direct + Distribution	- Safety aspects - a_w changes	~	✓
Sand (fluid)	+ Distribution + Handling	- Impact on tenacity?	+	✓
SiO_2 (fluid)	+ Distribution	- Dusty - Carrier effect?	~	-
Agar (fluid)	+ Distribution	- Impact on tenacity?	+	✓
Ready to eat food	*+ Distribution* *+ Handling*	*- Numerous impact factors*	+	✓

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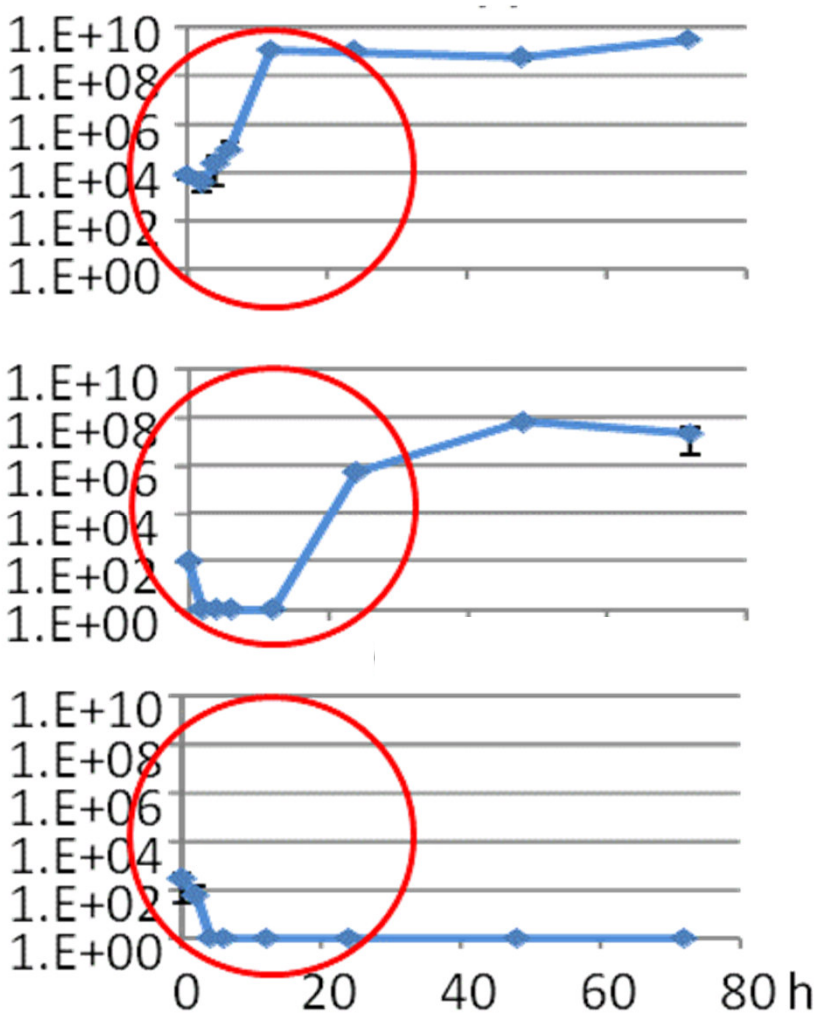
Detection

- **Adaptation and Optimization**
  - sample preparation
  - detection method
- **Comparison**
  - culture methods (ISO, national law)
  - molecular techniques (PCR)
- **Ring trial**



SPICED consortium; AGES

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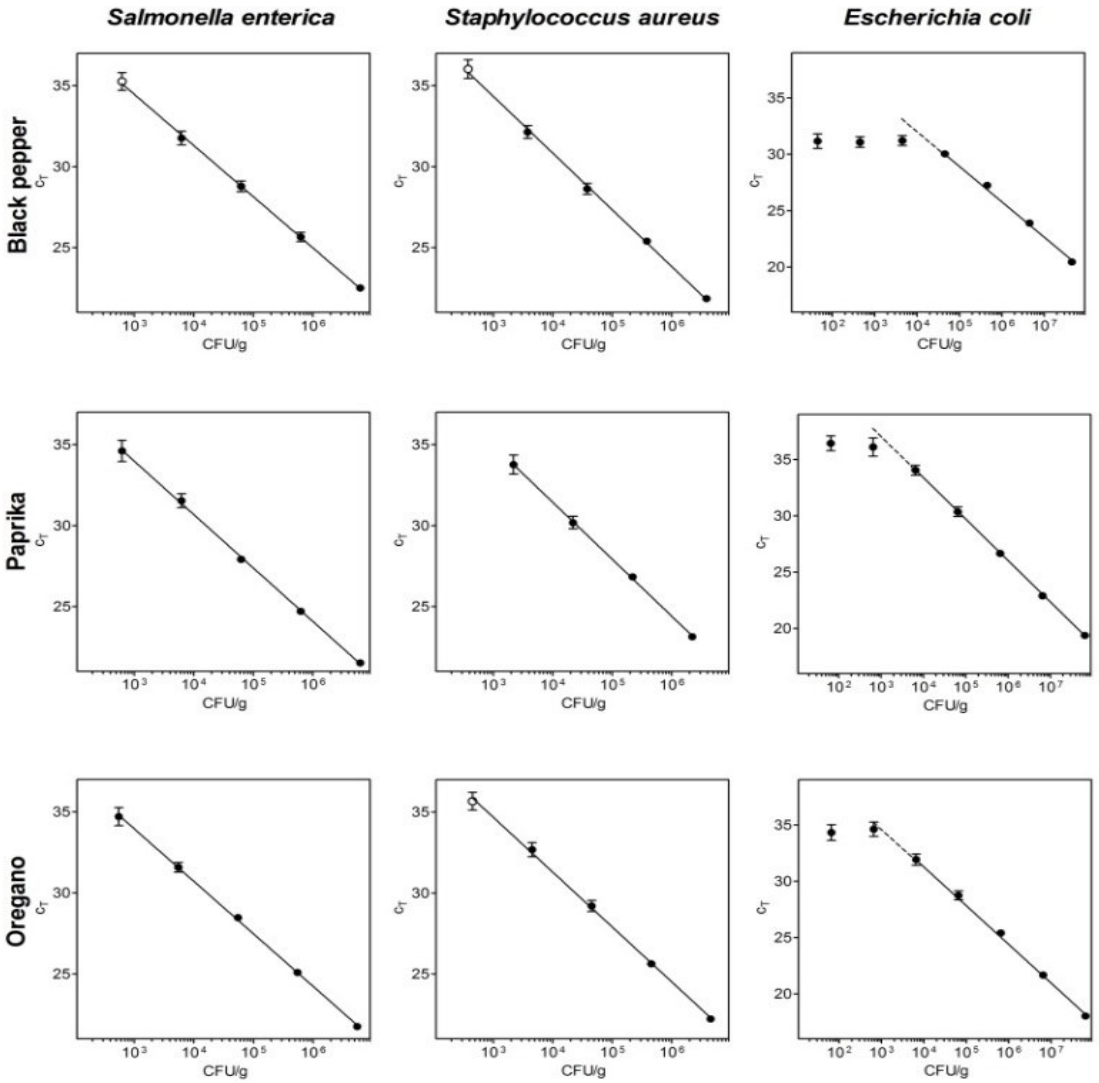
Culture-independent quantification of pathogens in spices and herbs



VUP

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Culture-independent quantification of pathogens in spices and herbs

	slope	*r*	LOQ (CFU/g)
S. enterica			
Black pepper	- 3.16	0.995	10^3
Paprika	- 3.30	0.995	10^2
Oregano	- 3.24	0.997	10^2
Staph. aureus			
Black pepper	- 3.50	0.995	10^3
Paprika	- 3.52	0.994	10^3
Oregano	- 3.39	0.996	10^3
E. coli			
Black pepper	- 3.20	0.996	10^4
Paprika	- 3.55	0.998	10^3
Oregano	- 3.48	0.995	10^3

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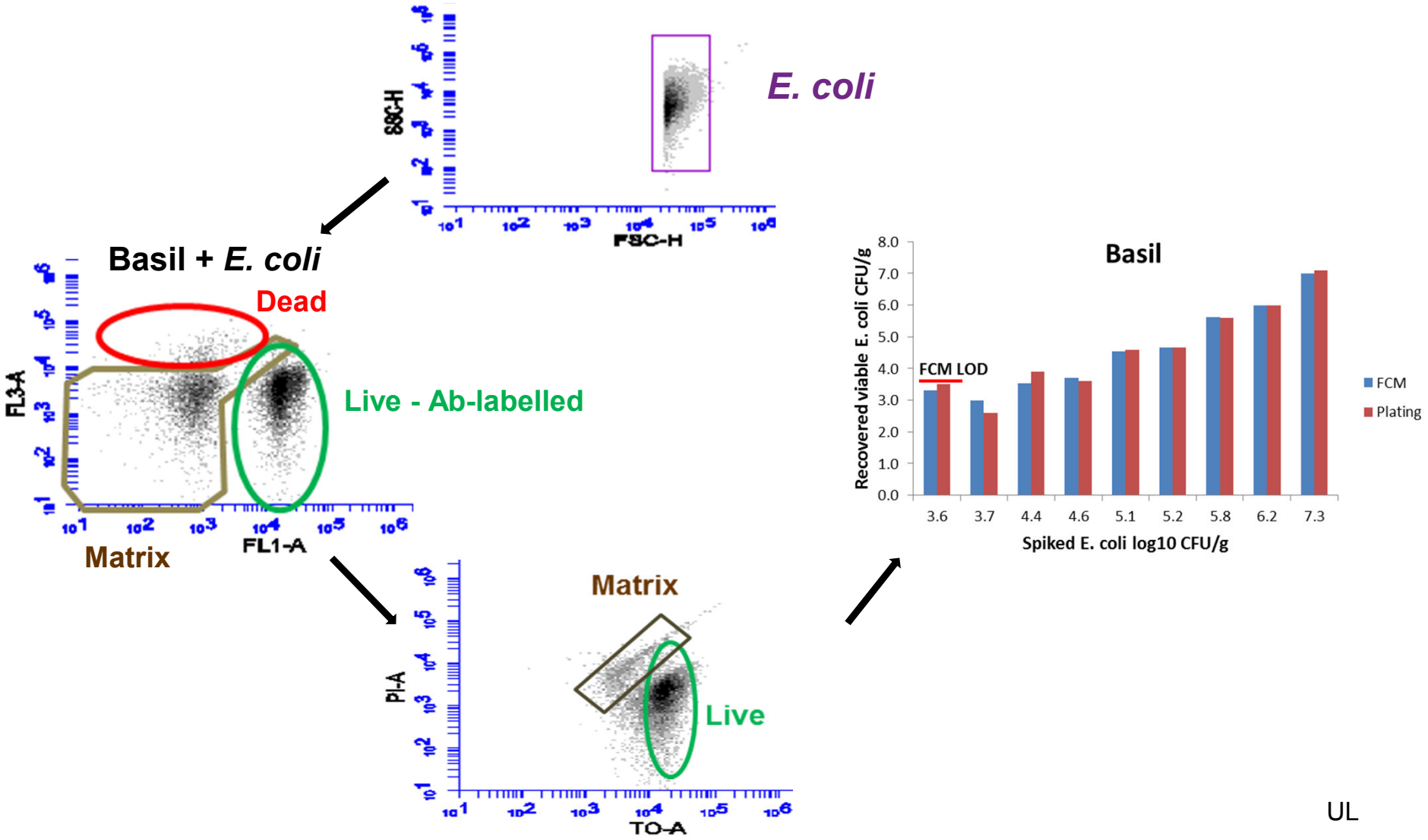
Differentiation of living and non-living bacteria



UL

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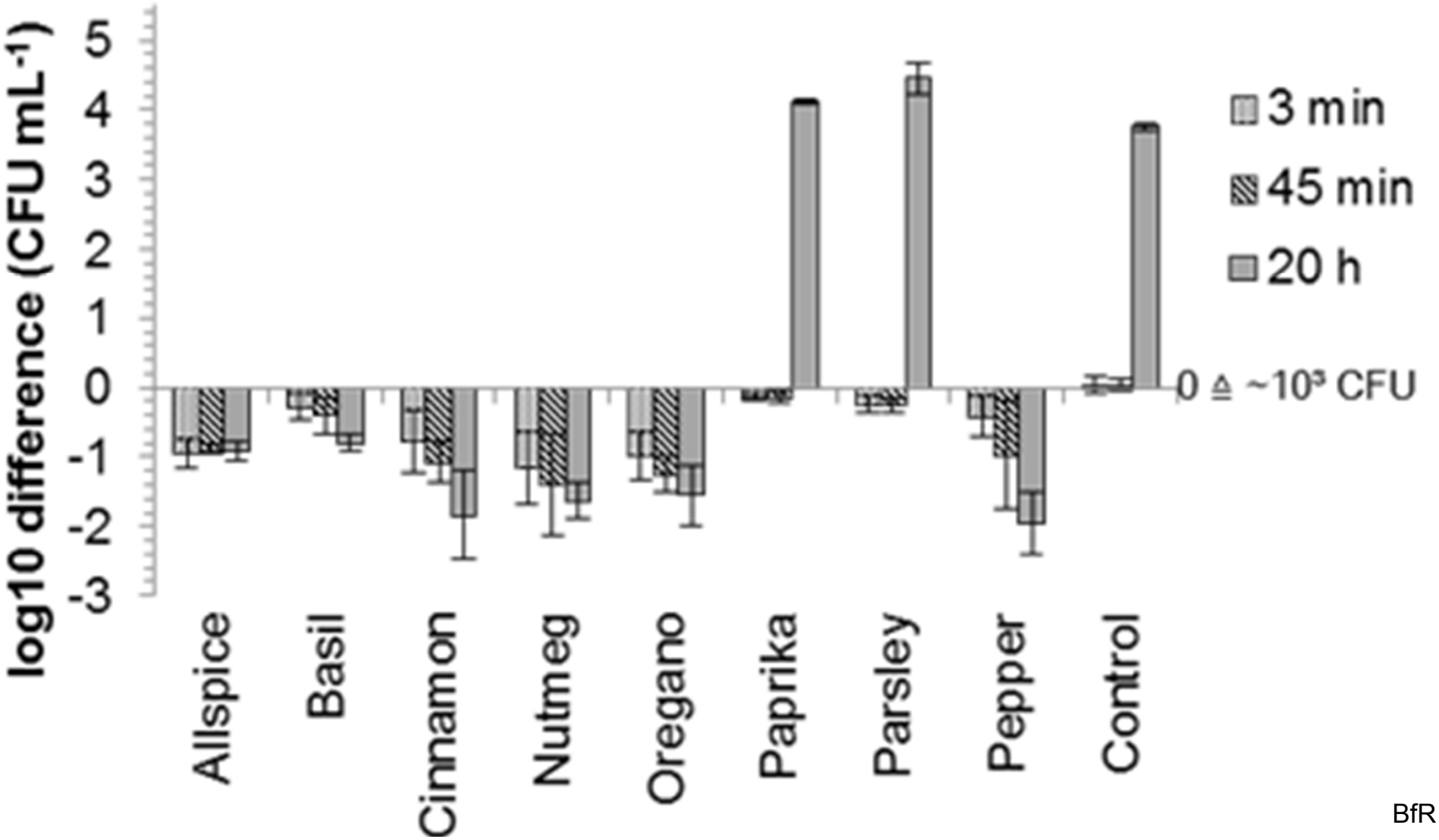
Inhibition of *Bacillus cereus* vegetative cells in spice and herb solutions



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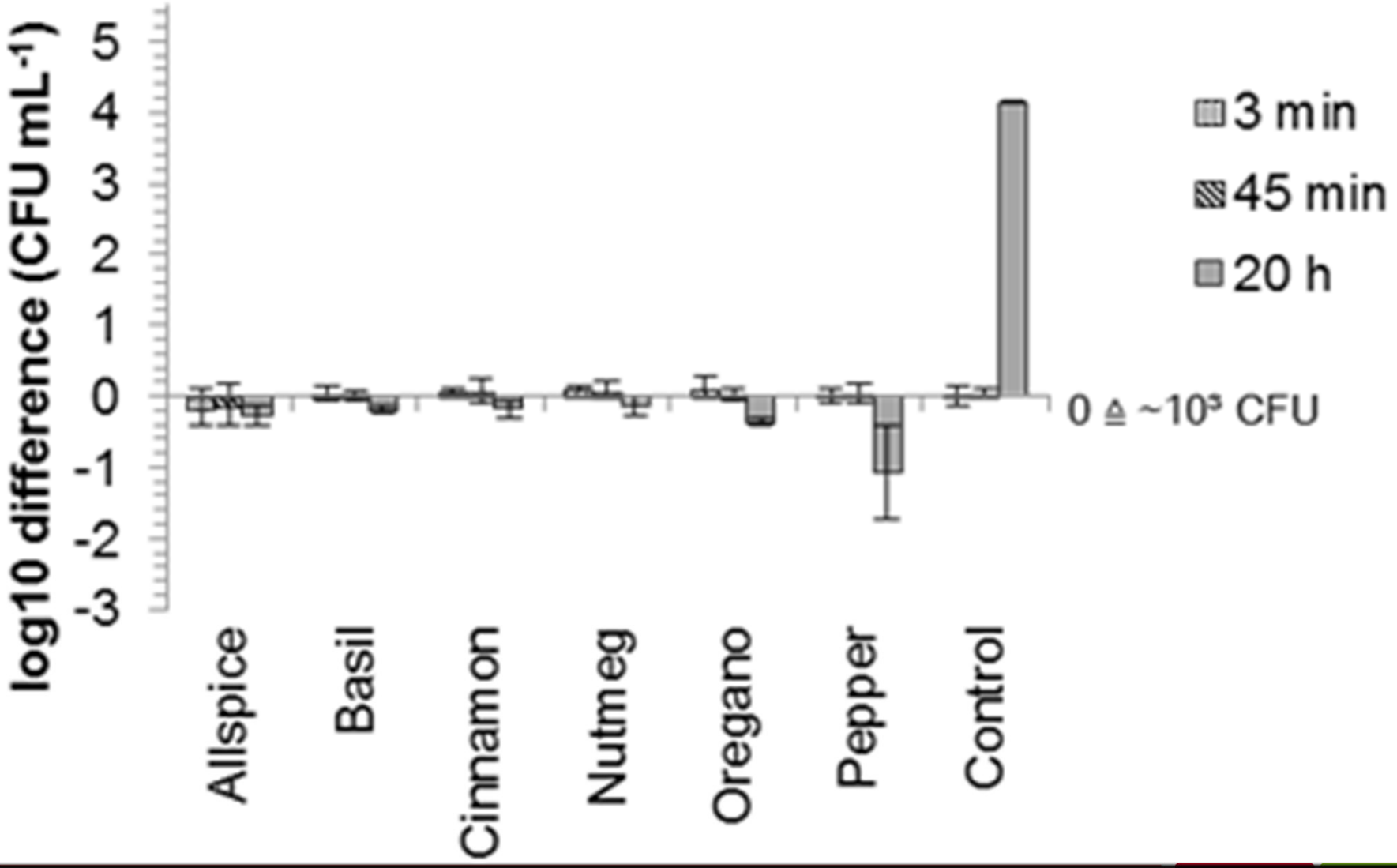
Inhibition of *Bacillus cereus* spores in spice and herb solutions



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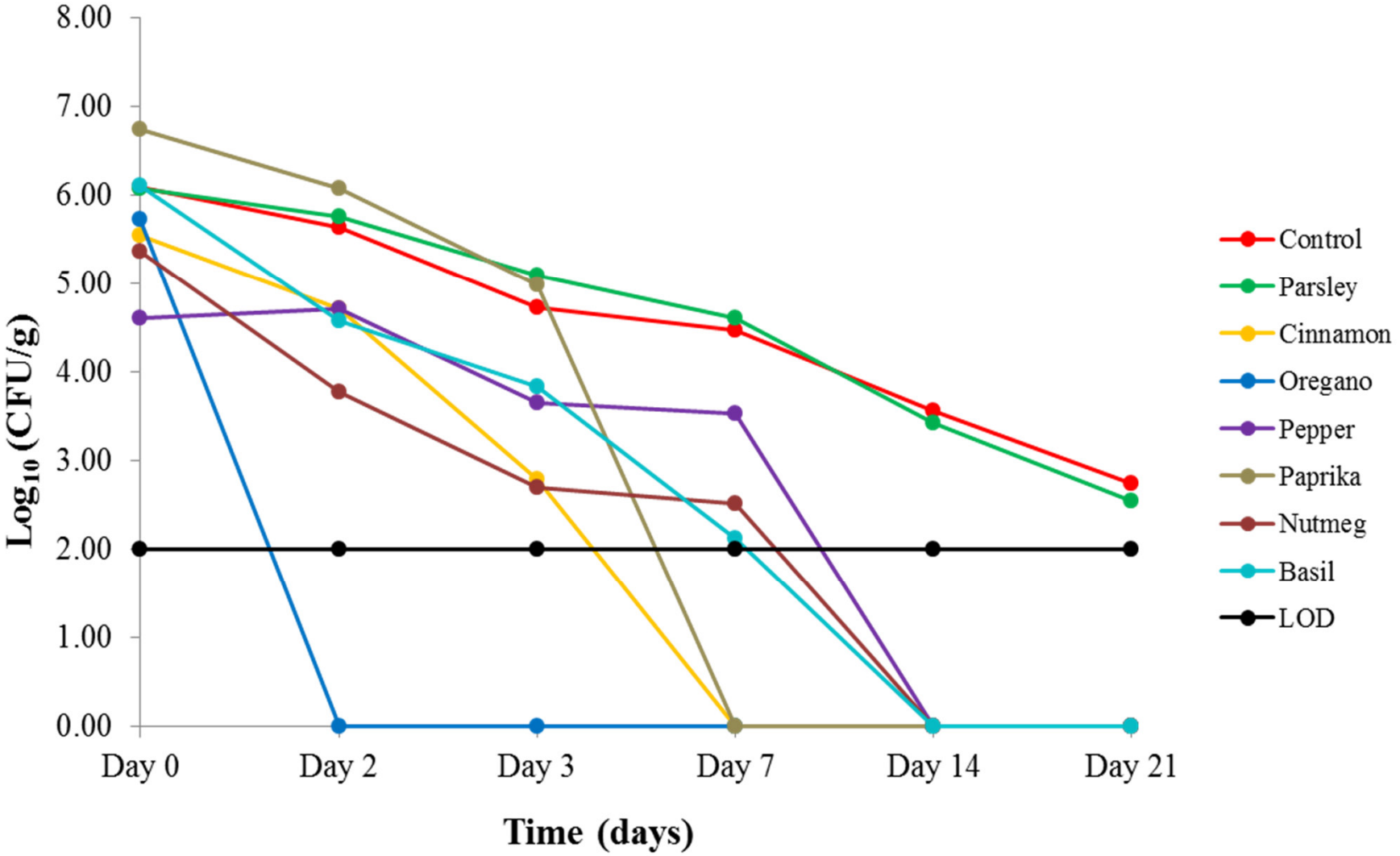
Tenacity of E. coli in spices and herbs



* *E. coli* **ATCC 11775**

** Storage at 25±1°C over 21 days

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Conclusion

- Improve detection of agents in spices and herbs
- Spiking techniques can influence tenacity
- Spices and herbs can affect tenacity
- Tenacity is depends on biological hazard and strain
- Tenacity data to be used for predictive microbiology

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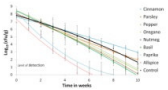
Biological agents in the focus

Agent	Diagnostic optimization 	Tenacity studies 	Ring trial 
Bacillus spp.	✓	✓	-
Brucella spp.	✓	✓	-
Clostridium perfringens	✓	✓	-
Escherichia coli	✓	✓	-
Listeria monocytogenes	✓	✓	-
Salmonella spp.	✓	✓	✓
Staphylococcus aureus	✓	✓	-
Ricin	✓	✓	-
SEB (Enterotoxin type B, produced by *Staphylococcus aureus)*	✓	✓	-

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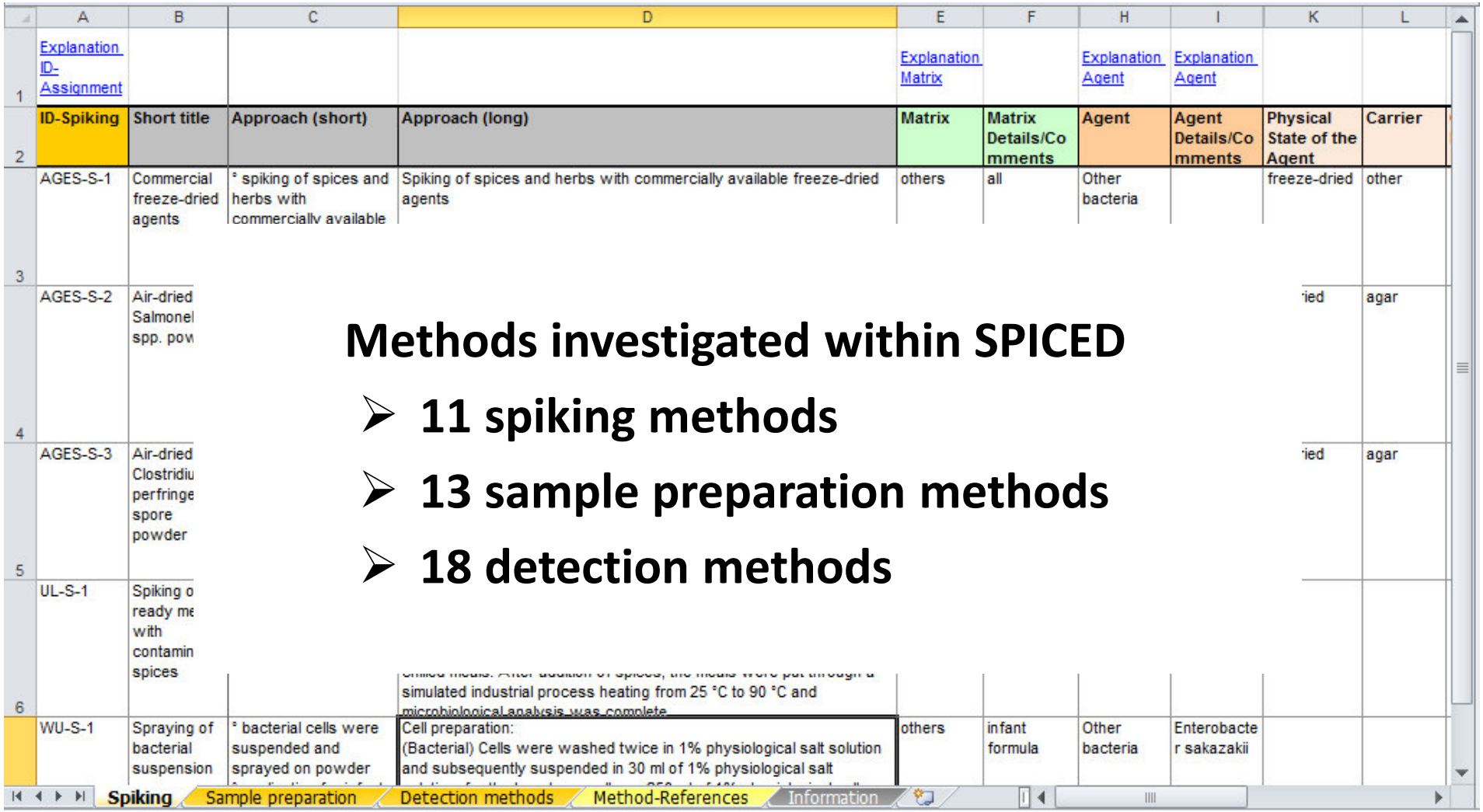
Diagnostic methods database



	A	B	C	D	E	F	H	I	K	L
1	Explanation ID-Assignment				Explanation Matrix		Explanation Agent	Explanation Agent		
2	ID-Spiking	Short title	Approach (short)	Approach (long)	Matrix	Matrix Details/Comments	Agent	Agent Details/Comments	Physical State of the Agent	Carrier
3	AGES-S-1	Commercial freeze-dried agents	° spiking of spices and herbs with commercially available	Spiking of spices and herbs with commercially available freeze-dried agents	others	all	Other bacteria		freeze-dried	other
4	AGES-S-2	Air-dried Salmonel spp. pow							ied	agar
5	AGES-S-3	Air-dried Clostridiu perfringe spore powder							ied	agar
6	UL-S-1	Spiking o ready me with contamin spices		simulated industrial process heating from 25 °C to 90 °C and microbiological analysis was complete						
	WU-S-1	Spraying of bacterial suspension	° bacterial cells were suspended and sprayed on powder	Cell preparation: (Bacterial) Cells were washed twice in 1% physiological salt solution and subsequently suspended in 30 ml of 1% physiological salt	others	infant formula	Other bacteria	Enterobacter sakazakii		

Spiking | Sample preparation | Detection methods | Method-References | Information

Methods investigated within SPICED

- 11 spiking methods
- 13 sample preparation methods
- 18 detection methods

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Thanks for your attention.
Questions and comments?

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